KISS1 and KISS1R expression in the human and rat carotid body and superior cervical ganglion

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Abstract

KISS1 and its receptor, KISS1R, have both been found to be expressed in central nervous system, but few data are present in the literature about their distribution in peripheral nervous structures. Thus, the aim of the present study was to investigate, through immunohistochemistry, the expression and distribution of *KISS1* and KISS1R in the rat and human carotid bodies and superior cervical ganglia, also with particular reference to the different cellular populations. Materials consisted of carotid bodies and superior cervical ganglia were obtained at autopsy from 10 adult subjects and sampled from 10 adult Sprague-Dawley rats. Immunohistochemistry revealed diffuse expression of *KISS1* and KISS1R in type I cells of both human and rat carotid bodies, whereas type II cells were negative. In both human and rat superior cervical ganglia positive anti-*KISS1* and -KISS1R immunostainings were also selectively found in ganglion cells, satellite cells being negative. Endothelial cells also showed moderate immunostaining for both *KISS1* and KISS1R. The expression of both kisspeptins and kisspeptin receptors in glomic type I cells and sympathetic ganglion cells supports a modulatory role of *KISS1* on peripheral chemoreception and sympathetic function. Moreover, local changes in blood flow have been considered to be involved in carotid body chemoreceptor discharge and kisspeptins and kisspeptin receptors have also been found in the endothelial cells. As a consequence, a possible role of kisspeptins in the regulation of carotid body blood flow and, indirectly, in chemoreceptor discharge may also be hypothesized.

Introduction

The *KISS1* gene was originally identified through a differential expression approach showing up regulation of its expression in tumor cells which had lost their potential to metastize.[1-3] It encodes for a 145 amino acid precursor, which can be cleaved into a 54 amino acid protein, originally called metastin, or shorter 14, 13 and 10 amino acid peptides, which share a common C-terminal amidation site. With the term of kisspeptins are collectively named all the peptides cleaved from the precursor hormone. The larger protein shows some variability among species whereas the C-terminal 10 amino acid sequence is well conserved.[1,4-6]

The kisspeptin receptor, now called KISS1R in humans and Kiss1r in rodents,[7] was initially discovered in rats in 1999 as an orphan G protein-coupled membrane receptor named GPR54,[8] in 2001 kisspeptins being identified as its natural ligands.[4-6,9] Its gene shares modest homology with the gene coding for the galanin receptor 2, although kisspeptins do not bind the above galanin receptor.[8]

Kisspeptins play a crucial role in the control of puberty onset and reproductive function. Apart from central nervous system, *KISS1* and KISS1R have both been found to be widely expressed in many other tissues such as the pituitary, gonads, placenta, pancreas, liver, intestines and vessels.[1,5,6,10,11] As it concerns the peripheral nervous system, both *KISS1* and KISS1R have recently been found to be expressed in rat dorsal root ganglion.[12] To the best of our knowledge, there are no data regarding expression of kisspeptin and kisspeptin receptor in the carotid body and sympathetic ganglia.

The carotid body is the main peripheral arterial chemoreceptor, inducing increases in ventilatory volume and frequency in response to hypoxia, hypercapnia, or reduction of blood pH. It is organized in lobules of cells belonging to two different populations: type I cells, with roundish shape and higher dimensions, and type II cells, with fusiform shape and located at the edges of the clusters. Type I cells represent the real chemoreceptor elements. In response to the various stimuli they release many different neurotransmitters and neuromodulators,[13-17] which mainly act on the glosso-pharyngeal afferent fibers arising from the petrosal ganglion. However, substances released from type I cells may also act on other components of the carotid body, such as type I cells themselves (through autocrine and paracrin mechanisms), type II cells, vessels, and connective cells. Type II cells show astrocytic markers and play a supportive role, but it has recently been observed that if exposed to prolonged hypoxia they may also behave as stem cells precursor for type I cells.[18] The carotid body also shows parasympathetic and sympathetic innervation, the latter mainly from the superior cervical ganglion.

The aim of the present study was therefore to investigate, through immunohistochemistry and real-time RT-PCR, the expression and distribution of *KISS1* and KISS1R in the rat and human carotid body and superior cervical ganglia, also with particular reference to the different cellular populations.

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Materials and Methods

Tissue sampling and preparation

Materials consisted of carotid bodies and superior cervical ganglia obtained at autopsy from 10 adult subjects [6 males, 4 females; mean age 46 years, standard deviation (SD)±3.6], clinically negative for chronic pulmonary or cardiovascular diseases, and sampled from 10 adult Sprague-Dawley rats. Autopsies were performed between 24 and 30 h after death.

Sampling from rats was performed soon after sacrifice. Right carotid bifurcations and superior cervical ganglia from humans and rats were fixed in Bouin solution and embedded in paraffin wax.

Immunohistochemical analysis

Immunohistochemical examinations were carried out on 3 μm thick sections. For anti-KISS1R immunohistochemistry, unmasking was performed with 10 mM sodium citrate buffer, pH 6.0, at 90°C for 30 min. For anti-*KISS1* immunohistochemistry, antigen unmasking was not necessary. Sections were incubated in 0.03% hydrogen peroxide for 10 min at room temperature, to remove endogenous peroxidase activity, and then in blocking serum (0.04% bovine serum albumin, A2153, Sigma-Aldrich, Milan, Italy and 0.5% normal goat serum X0907, Dako Corporation, Carpinteria, CA, USA, in PBS) for 30 min at room temperature. Primary anti-*KISS1* antibody (rabbit polyclonal antibody anti-metastin [1-25)/KISS-1 (68-92), Catalog No. H-048-62, Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA] was diluted 1:1500 in blocking serum. Primary anti-KISS1R antibody (rabbit polyclonal antibody anti-AXOR12 (375-398), Catalog No. H-048-61, Phoenix Pharmaceuticals, Inc.,) was diluted 1:100 in blocking serum. Both antibodies were incubated overnight at 4°C. Sections were then washed three times for 5 min in PBS. Sections were revealed with anti-rabbit serum (DAKO® EnVision + TM Peroxidase, Rabbit, Dako Corporation) for 30 min at room temperature. Finally, sections were developed in 3,3'-diaminobenzidine (DAB, Sigma-Aldrich) and counterstained with hematoxylin. Negative controls were performed by omission of primary antibody and absorption tests. Immunoreactions detected in human placenta and rat brain were used as positive controls.

The percentages of type I and II positive cells were evaluated on fields of 180×134 μm. Five sections and 3 fields per section were examined. In rat and human carotid bodies, about 40 and 25-30 type I cells were counted for each field, respectively. The mean percentages of positive cells were calculated for each case and for the entire series.

Statistics

Percentages obtained were compared with Mann-Whitney test. A P value of 0.05 was considered significant.

Results

Immunohistochemistry revealed diffuse expression of kisspeptins and receptor in type I cells of both human and rat carotid bodies. Immunostained cells were distributed both in the centre and in the periphery of the lobules. Conversely, no kisspeptins or receptor immunostainings were observed in type II cells (Figure 1). Endothelial cells of some vessels also showed moderate immunostaining for both *KISS1* and KISS1R.

In both human and rat superior cervical ganglia positive anti-*KISS1* and –KISS1R immunostainings were selectively found in ganglion cells. Satellite cells were negative or only showed weak reaction (Figure 2). Glomic and ganglion cell immunostainings were eliminated when preabsorbed antiserum was used or primary antibodies were omitted.

Discussion

To the best of our knowledge, this is the first study demonstrating kisspeptin and kisspeptin receptor expression in glomic type I cells through immunohistochemistry. In the carotid body, glomic type I cells release many neurotransmitters and peptide neuromodulators that play a role in the regulation of chemoreceptor

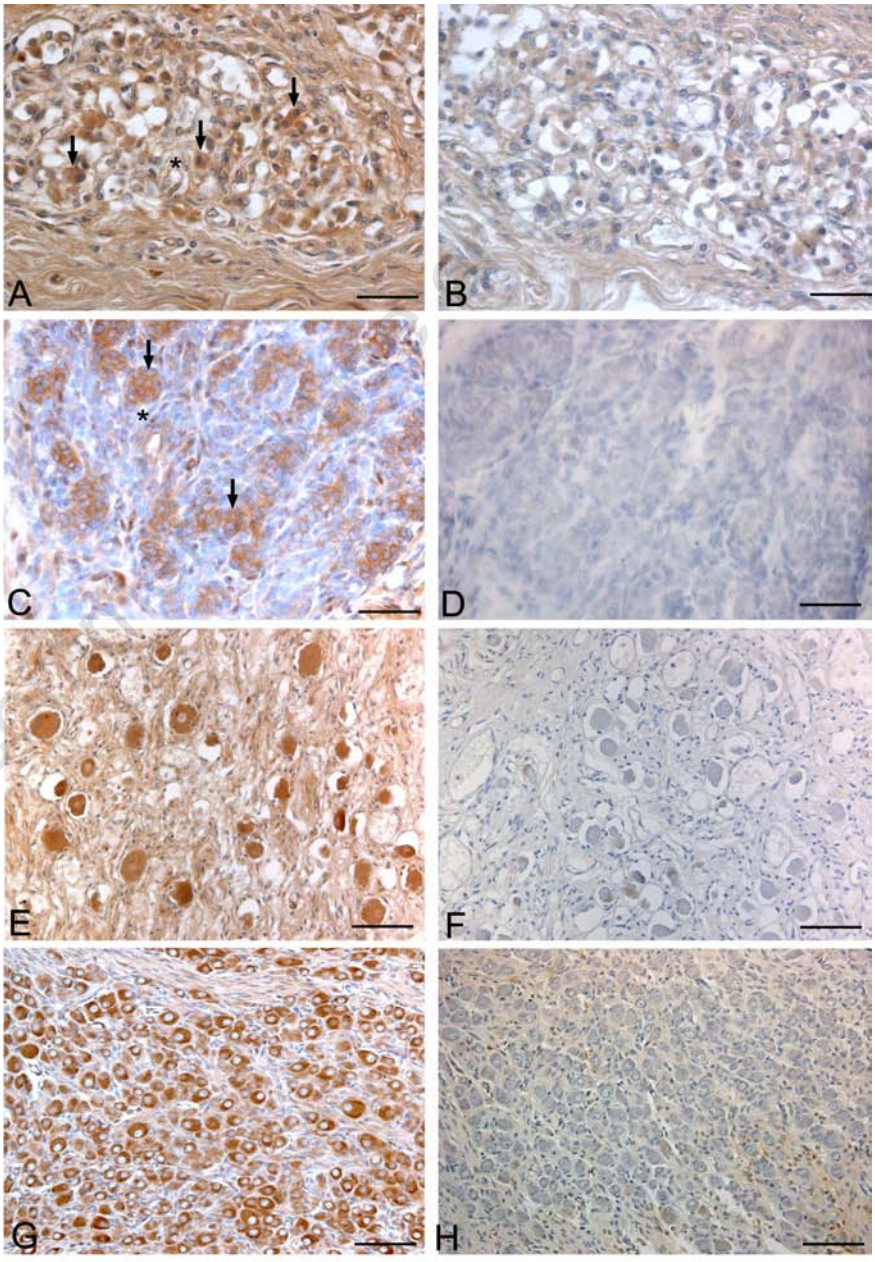

Figure 1. Anti-*KISS1* immunohistochemistry in human (A,B,E,F) and rat (C,D,G,H) carotid bodies (A-D) and superior cervical ganglia (E-H), showing selective positivity of glomic type I cells (arrows) and ganglionic cells, immunostaining being largely eliminated in negative controls (B,D,F,H). Note also negativity of type II cells (asterisks) in the carotid body and of satellite cells in the superior cervical ganglia. Scale bars: A-D, 37.5 μm; E-H, 75 μm.

discharge.[13-15,19,20] Our findings about expression of both *KISS1* and KISS1R in type I cells, which represent the real chemoreceptive element of the carotid body, support a modulatory role of *KISS1* on peripheral chemoreception. Kisspeptins have been found to directly stimulate excitability of neurons, such as the granule cells of the dentate gyrus,[21] and to modulate the release of other peptides, such as in hypophysis[22] and islets of Langherans.[23,24] Thus, also in the carotid body kisspeptins could act on excitability of type I cells and/or on their release of peptide neuromodulators. Further functional studies will be necessary to clarify the effects of kisspeptins on chemosensory process in glomic cells. Kisspeptin receptor probably preferentially bind peptides locally released in the carotid body but circulating factors have also been reported to act on carotid body type I cells, and possible actions by blood-borne kisspeptins may not be excluded.

The possible role of kisspeptins in the cardiovascular system has also recently been considered. The carotid body is the structure in the body with the highest blood flow (about 1400 or 2000 mL/100 g/min in the cat, depending on the technique for determination of tissue blood flow),[25,26] and local changes in blood flow have been considered to be involved in carotid body chemoreceptor discharge.[20,27,28] KISS1R and *KISS1* have been found to be expressed in the endothelial cells of the aorta, coronary artery and umbilical vein and kisspeptins have been demonstrated to show potent vasoconstrictor action.[10] In the present work anti-*KISS1* and – KISS1R immunoreactions were also found in endothelial cells of some vessels, as a consequence a possible role of kisspeptins in the regulation of carotid body blood flow and, indirectly, in chemoreceptor discharge may be hypothesized.

Kisspeptins could also show trophic actions on glomic cells. In the pre- and postnatal periods the carotid body undergoes structural changes, mediated by trophic factors, including carotid body volume increase, proliferation of type I, type II, endothelial and Schwann cells lining peripheral nerve fibers and increased number of synapses between type I and II cells.[15,29] Moreover, environmental stimuli (for instance, hypoxic, hyperoxic or inflammatory noxae) may also cause a series of morphological, cellular and biochemical changes.[29-34] For instance, chronic hypoxia has been shown to increase O_2 sensitivity in the carotid body through changes in molecular chemoreceptors, ion channels and neurochemicals.[35-37] The above changes are mediated by a wide series of trophic factors. It may also be hypothesized that kisspeptins released by type I cells may modulate the expression and effects of trophic factors in the carotid body. For instance, kisspeptins have been found to increase BDNF expression in hippocampal slice cultures,[38] and this growth factor has been identified in type I cells and nerve fibers of rat carotid body.[15,39,40]

In the literature, *KISS1* and KISS1R have been found to be expressed in the dorsal root ganglia and to be upregulated after intraarticular injection of the complete Freund's adjuvant, suggesting possible involvement in chronic inflammatory pain.[12] In the present work, expressions of both *KISS1* and KISS1R were found in ganglion cells of the human and rat superior cervical ganglia. Thus, it may be hypothesized a role of kisspeptins also in the modulation of the sympathetic function, probably mainly through autocrine or paracrine mechanisms.

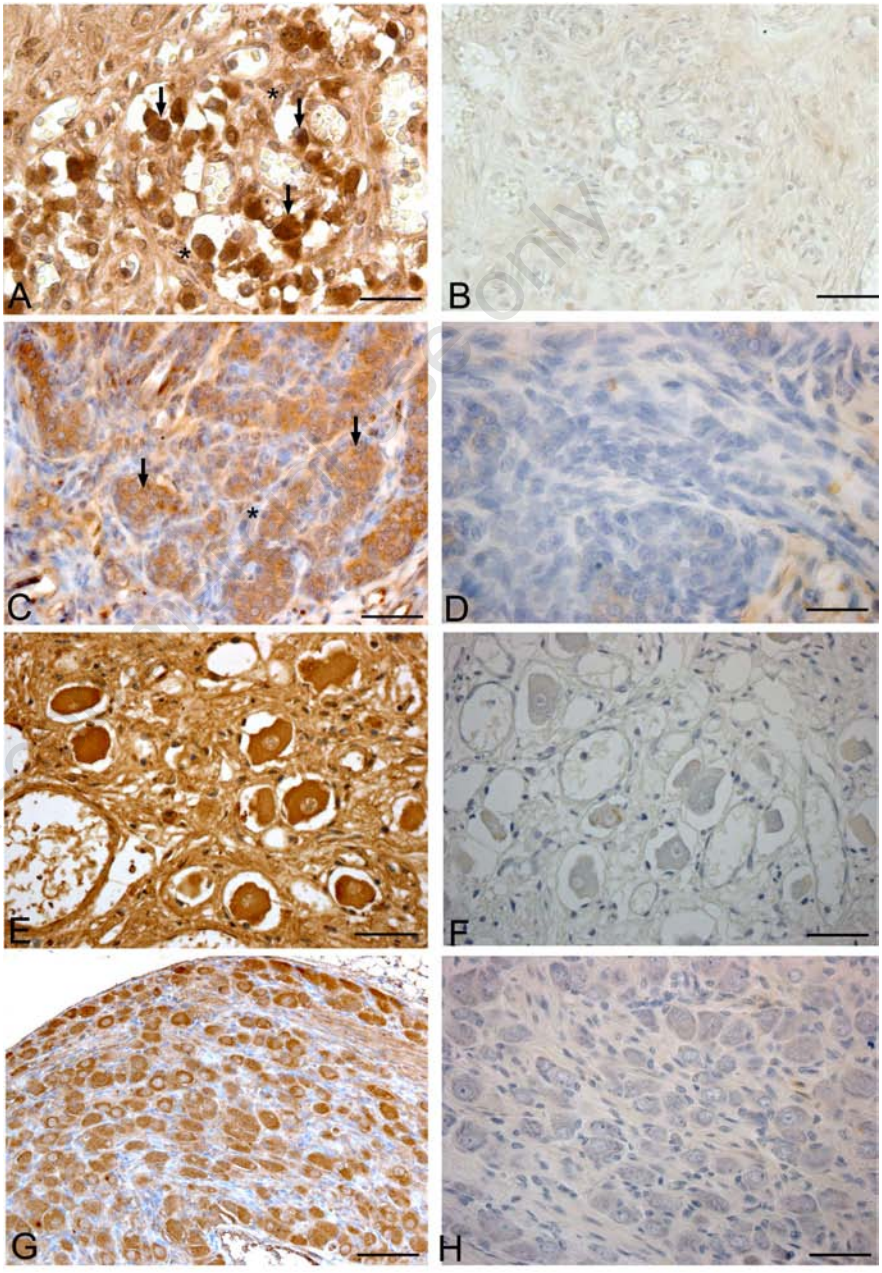

Figure 2. Anti-KISS1R immunohistochemistry in human (A,B,E,F) and rat (C,D,G,H) carotid bodies (A-D) and superior cervical ganglia (E-H), showing selective positivity of glomic type I cells and ganglionic cells, immunostaining being largely eliminated in negative controls (B,D,F,H). Note also negativity of type II cells (asterisks) in the carotid body and of satellite cells in the superior cervical ganglia. Scale bars: A, D, 23.8 μm; B, C, E, F, 37.5 μm; G-H, 75 μm.

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